A Convenient Synthesis of Hispidin from Piperonal

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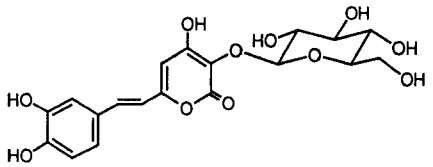
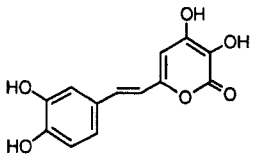
An improved synthesis of hispidin (overall yield 24%) starting from the commercially available piperonal is reported. This three-step method is more effective and advantageous compared to the known six-step preparation.

Styrylpyrones are natural products accumulated in fungi[1] and higher plants.[2] The first styrylpyrone glycoside, namely *equisetumpyrone* **A**, was isolated from gametophytes and fertile sprouts of *Equisetum arvense L.*, whose structure was recently elucidated.[3] Its biosynthesis is still unknown and we propose that this styrylpyrone glycoside is formed through caffeoyl-CoA, which combines with malonate, and by hydroxylation of the resulting hispidin and subsequent glucosilation of the hydroxyhispidin **B**. Hispidin, a naturally occurring styrylpyrone, whose structure was determined as 4-hydroxy-6-(3′,4′-dihydroxystyryl)-2-pyrone **(4)**[4] was first isolated from *Polyporus hispidus* in 1889.[5] This biologically active trihydroxy-substituted styrylpyrone exhibits *in vitro* antimicrobial activity against Gram-positive organisms, and also the acid-fast *Mycobacterium smegmatis*.[6]



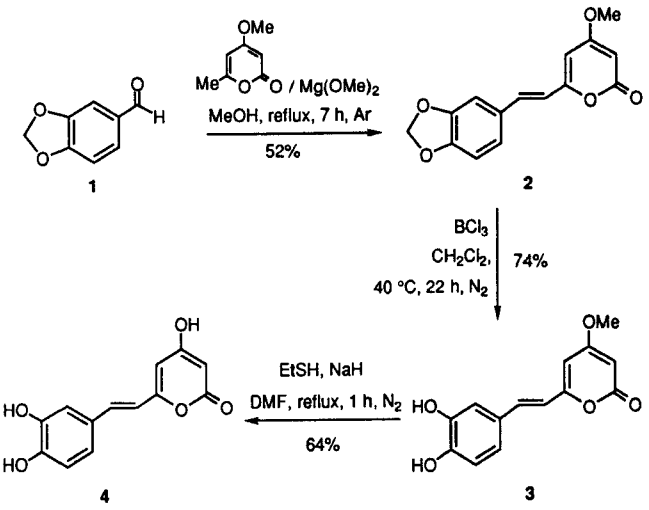
A



B

To verify the proposed biosynthetic route of *equisetumpyrone*, we required sufficient amounts of hispidin. A cumbersome, multistep synthesis of hispidin was reported earlier;[7] unfortunately, we encountered difficulties with this synthetic sequence and were, therefore, obliged to work out a more convenient method. We report here a three-step synthesis of hispidin **(4)** (24% overall yield), which starts from the commercially available piperonal **(1)**, as displayed in the Scheme.

The condensation of piperonal **(1)** with 4-methoxy-6-methyl-2-pyrone[8] in the presence of magnesium methoxide, analogous to the reported preparation of 4-methoxy-6-(4′-methoxystyryl)-2-pyrone,[8] afforded the methylenedioxy styrylpyrone **2** in 52% yield. The selective demethylenation of **2** with boron trichloride in dichloromethane at 40 °C gave the dihydroxy styrylpyrone **3** in 74% yield. The latter was converted to hispidin **(4)**



Scheme

in 64% yield by demethylation of the pyrone ether group with sodium thioethoxide in dimethylformamide at reflux.

The advantages of the present three-step route (Scheme) over the previous six-step one are:
a) instead of 3,4-di(methoxymethoxy)benzaldehyde, which is prepared by alkylation of 3,4-dihydroxybenzaldehyde with expensive and toxic chloromethyl methyl ether, commercially available piperonal **(1)** is employed as the carbonyl component for condensation with methylpyrone, which proceeds in much better yield (52% vs. 23%), and b) the methylenedioxy group of **2** is readily cleaved by boron trichloride to release the dihydroxy-substituted styrylpyrone methyl ether **3**, which is effectively demethylated to hispidin **(4)** by sodium thioethoxide in dimethylformamide,[9] an ether cleaving reagent employed for the first time for such an application. In contrast, several steps are required for the deprotection of 4-methoxy-6-[3′,4′-di(methoxymethoxy]-styryl-2-pyrone to hispidin, which was employed in the literature procedure.[7]

Solvents were purified according to standard procedures. CH_2Cl_2 was distilled from P_2O_5 and DMF from CaH_2. Melting points were determined on a Reichert Thermovar hot stage apparatus. IR spectra were recorded on a Perkin-Elmer 1420 spectrometer and the UV spectra on a Hitachi U-3200 spectrophotometer. ^1H and ^{13}C NMR spectra were acquired on a Bruker AC 200 (200 MHz) or AC 250 (250 MHz) spectrometer. Chemical shifts refer to $CDCl_3$, DMSO-d_6, or CD_3OD. The FAB mass spectrum was obtained on a Finnigan MAT 8430 instrument by using glycerol as matrix. 4-Methoxy-6-methyl-2-pyrone was prepared according to the literature procedure by methylation of the commercially available 4-hydroxy-6-methyl-2-pyrone with dimethyl sulfate.[8]

4-Methoxy-6-(3′,4′-methylenedioxystyryl)-2-pyrone (2):
To a suspension of $Mg(OMe)_2$ (60.0 mmol, prepared from 1.46 g

of Mg turnings) in anhydr. MeOH (40 mL) was added dropwise a solution of piperonal (**1**; 3.00 g, 20.0 mmol) and 4-methoxy-6-methyl-2-pyrone (3.36 g, 24.0 mmol) in MeOH (40 mL) under an Ar atmosphere. The mixture was stirred under gentle reflux for 7 h, the solvent was removed by rotoevaporation (40 °C/20 Torr), the residue was dissolved in CH_2Cl_2 (25 mL) and treated with 3.3 M AcOH (30 mL) in H_2O. The aqueous layer was separated and extracted with CH_2Cl_2 (5 × 25 mL). The combined organic layers were washed with H_2O (6 mL), sat. $NaHCO_3$ solution (12 mL) and brine (6 mL), and dried (Na_2SO_4). After removal of the solvent (20 °C/20 Torr) the residue was recrystallized from MeOH (1 L) to afford 2.82 g (10.4 mmol, 52%) of styrylpyrone **2** as pale yellow needles; mp 234–235 °C (Lit.[10] mp 236 °C).

IR (KBr): ν = 3070, 1715, 1625, 1605, 1550, 1505, 1440, 1405, 1250, 1155, 1030 cm^{-1}.

UV (MeOH): λ_{max} (log ε) = 223 (4.284), 251 (3.993), 363 nm (4.325).

^{1}H NMR ($CDCl_3$, 250 MHz): δ = 3.82 (s, 3 H, OCH_3), 5.47 (d, J = 2.2 Hz, 1 H, 3-H), 5.89 (d, J = 2.2 Hz, 1 H, 5-H), 5.99 (s, 2 H, OCH_2O), 6.40 (d, J = 15.9 Hz, 1 H, 8′-H), 6.80 (d, J = 8.0 Hz, 1 H, 5′-H), 6.97 (dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 1 H, 6′-H), 7.00 (d, J = 1.5 Hz, 1 H, 2′-H), 7.41 (d, J = 15.9 Hz, 1 H, 7′-H).

4-Methoxy-6-(3′,4′-dihydroxystyryl)-2-pyrone (3):

A 1 M solution of BCl_3 in hexane (6.91 mL, 6.91 mmol) was added dropwise to a solution of styrylpyrone **2** (0.607 g, 2.23 mmol) in anhydr. CH_2Cl_2 (100 mL) under an N_2 atmosphere. Stirring was continued for 22 h at reflux and MeOH (10 mL) was added at 20 °C to the mixture. The solvent was removed (20 °C/20 Torr) and the residue was recrystallized from MeOH (25 mL) to give 0.431 g (1.65 mmol, 74%) of styrylpyrone **3** as yellow needles, mp 256–257 °C (Lit.[7] mp 257 °C).

IR (KBr): ν = 3600–2900, 1645, 1615, 1585, 1530, 1440, 1395, 1335, 1285, 1240, 1135, 1095, 1025, 950 cm^{-1}.

UV (MeOH): λ_{max} (log ε) = 220 (4.372), 253 (4.133), 371 nm (4.405).

^{1}H NMR (DMSO-d_6, 200 MHz): δ = 3.81 (s, 3 H, OCH_3), 5.58 (d, J = 2.1 Hz, 1 H, 3-H), 6.25 (d, J = 2.1 Hz, 1 H, 5-H), 6.66 (d, J = 16.0 Hz, 1 H, 8′-H), 6.76 (d, J = 8.2 Hz, 1 H, 5′-H), 6.94 (dd, J_1 = 8.2 Hz, J_2 = 1.7 Hz, 1 H, 6′-H), 7.03 (d, J = 1.7 Hz, 1 H, 2′-H), 7.15 (d, J = 16.0 Hz, 1 H, 7′-H).

^{13}C NMR (DMSO-d_6, 50 MHz): δ = 56.2 (q, CH_3), 87.9 (d, C-3), 100.0 (d, C-5), 114.0 (d, C-2′), 115.7 (d, C-5′), 116.0 (d, C-8′), 120.3 (d, C-6′), 126.6 (s, C-1′), 134.7 (d, C-7′), 145.5 (s, C-3′), 147.4 (s, C-4′), 158.9 (s, C-6), 162.7 (s, C-2), 170.9 (s, C-4).

Hispidin (4):

To a suspension of NaH (72.0 mg, 60% oil dispersion) in anhydr. DMF (1 mL) was added a solution of EtSH (107 mg, 1.73 mmol) in DMF (2 mL) under an N_2 atmosphere. The mixture was stirred for 5 min and a solution of styrylpyrone **3** (100 mg, 0.384 mmol) in DMF (3 mL) was added. Stirring was continued for 1 h at reflux, the mixture was cooled to 0 °C, acidified (pH 6) with 10% aq HCl and extracted with EtOAc (5 × 5 mL). The combined extracts were dried (Na_2SO_4) and the solvent removed (40 °C, 20 Torr). The crude product was recrystallized from aq MeOH to yield 60.0 mg (0.244 mmol, 64%) of hispidin **(4)** as yellow needles, mp 258–259 °C (dec.) (Lit.[6] mp 259 °C).

MS (FAB, Glycerol): m/z = 247 $(M + H)^+$.

IR (KBr): ν = 3600–2900, 1635, 1585, 1530, 1425, 1355, 1275, 1250, 1185, 1140, 1100, 950 cm^{-1}.

UV (MeOH): λ_{max} (log ε) = 220 (4.363), 252 (4.116), 369 nm (4.380).

^{1}H NMR (CD_3OD, 200 MHz): δ = 5.37 (d, J = 1.9 Hz, 1 H, 3-H), 6.07 (d, J = 1.9 Hz, 1 H, 5-H), 6.53 (d, J = 15.9 Hz, 1 H, 8′-H), 6.75 (d, J = 8.2 Hz, 1 H, 5′-H), 6.89 (dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz, 1 H, 6′-H), 7.01 (d, J = 1.9 Hz, 1 H, 2′-H), 7.25 (d, J = 15.9 Hz, 1 H, 7′-H).

^{13}C NMR (CD_3OD, 50 MHz): δ = 90.3 (d, C-3), 101.7 (d, C-5), 114.8 (d, C-2′), 116.5 (d, C-5′), 116.8 (d, C-8′), 122.0 (d, C-6′), 128.7 (s, C-1′), 137.2 (d, C-7′), 146.6 (s, C-3′), 148.6 (s, C-4′), 161.9 (s, C-6), 167.7 (s, C-2), 173.3 (s, C-4).

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